INFECTION AND TRANSMISSION STUDIES WITH EASTERN ENCEPHALITIS VIRUS AND *ANOPHELES ALBIMANUS* AND *A. QUADRIMACULATUS*

WILLIAM E. COLLINS, ANDREW J. HARRISON AND JOHN R. JUMPER
National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Parasite Chemotherapy, P. O. Box 190, Chamblee, Georgia

Attempts to transmit eastern encephalitis virus (EEE) through *Anopheles* mosquitoes have been reported by Ten Broeck and Merrill (1935), Davis (1940) and Chamberlain *et al.* (1954). The species tested, *Anopheles quadrimaculatus, A. punctipennis* and *A. crucians,* all failed to transmit EEE virus by conventional methods, even though the virus could be maintained for extended periods in these mosquitoes.

The use of a membrane feeding technique to determine infection and transmission thresholds using combinations of *Anopheles* mosquitoes and Semliki Forest virus and *Aedes aegypti* and EEE virus has been described by Collins *et al.* (1964, 1965). In the present investigation, this membrane feeding technique has been used for the determination of infection and transmission thresholds for EEE virus in *A. albimanus* and *A. quadrimaculatus.*

MATERIALS AND METHODS. The eastern encephalitis virus (EEE), strain NJO-60, was obtained through the courtesy of Dr. Telford Work, Communicable Disease Center, Atlanta, Georgia.

The *A. albimanus* mosquitoes were the A-9 strain originally obtained from San Salvador and maintained in our laboratory since 1960.

The *A. quadrimaculatus* mosquitoes were the Q-1 strain which was obtained from Technical Development Laboratories, CDC, Savannah, Georgia, and maintained in our laboratory since 1959.

Mosquitoes were infected by allowing them to feed through a Baudruche (untreated) membrane on serial 10-fold dilutions of EEE virus in fresh heparinized rabbit blood. The virus suspensions were prepared by grinding in a mortar the brains of six moribund mice in four milliliters of Bacto heart infusion broth (Difco) and centrifuging for 15 minutes at 1500 r.p.m. Serial 10-fold dilutions of the supernatant were made in broth. For the mosquito feeding, one part of each dilution was added to four parts of blood; this was then warmed to 37° C. and placed on the membrane which formed the bottom of a half-pint ice cream cup. The cup was then placed on top of the cage containing the mosquitoes. The feeding period was 15 minutes, after which time the engorged mosquitoes were transferred to holding cages in an incubator at 25° to 26° C. The mosquitoes were fed 5 percent Karo solution daily on a cellulose pledget.

After 7 to 13 days of extrinsic incubation, mosquitoes were allowed to feed individually on wet baby chicks. Approximately 24 hours later, blood samples were drawn by cardiac puncture and the blood inoculated intracerebrally into five weanling mice. Presence of virus in the chick blood, as indicated by the death of the mice from EEE virus, constituted evidence of virus transmission by the mosquito.

To determine virus titers, mosquitoes were killed by freezing immediately after their initial feeding and after transmission attempts. These were stored in a mechanical freezer at −65° to −70° C. until titrated. Mosquitoes were ground individually in a mortar with a 1 ml. aliquot of Bacto-heart infusion broth containing 1000 units of penicillin and 2 milligrams of streptomycin per ml. The suspension was centrifuged for 15 min-

TABLE 1.—Relationship between eastern encephalitis virus ingested by *Anopheles albimanus* mosquitoes and that present after 7 and 11 days of extrinsic incubation.

Initial			Post Incubation				
Pos./ Tested	Pos. EEE Virus Titers *	Median Pos. EEE Titer	Day	Pos./ Tested	Percent Infected	Pos. EEE Virus Titers	Median Pos. EEE Titer
5/5	8.1, 8.0, 8.0, 8.0, 7.0	8.0	7	14/14	100	6.2, 6.0, 6.0, 6.0, 6.0, 5.9, 5.8, 5.5, 5.5, 5.2, 5.2, 4.5, 3.1, 2.5	5.8
			11	18/19	95	7.2, 6.8, 6.5, 6.5, 6.3, 6.2, 6.2, 6.1, 6.0, 6.0, 5.9, 5.8, 5.1, 4.8, 4.7, 4.3, 3.9, 3.3	6.0
5/5	7.0, 7.0, 6.9, 6.7, 6.5	6.9	11	2/11	18	5.7, 5.2	5.5
5/5	6.0, 6.0, 6.0, 5.5, 5.5	6.0	11	2/11	18	5.7, 5.7	5.7
5/5	5.0, 4.8, 4.7, 4.5, 3.1	4.7	11	1/12	8	5.0	5.0
5/5	4.3, 4.2, 4.1, 4.0, 3.9	4.1	11	0/11	0	..	..

* All titers expressed as the mouse $1/\log_{10}$ IC LD_{50}.

TABLE 2.—Relationship between eastern encephalitis virus ingested by *Anopheles quadrimaculatus* mosquitoes and that present after 10 to 13 days of extrinsic incubation.

Initial			Post Incubation				
Pos./ Tested	Pos. EEE Virus Titers *	Median Pos. EEE Titer	Day	Pos./ Tested	Percent Infected	Pos. EEE Virus Titers	Median Pos. EEE Titer
10/10	8.2, 8.0, 7.9, 7.8, 7.7, 7.5, 7.5, 7.3, 7.2, 7.0	7.6	10	8/20	40	5.5, 5.4, 5.3, 5.0, 4.5, 4.3, 4.2, 3.8	4.8
			11	6/12	50	7.7, 6.2, 5.7, 5.5, 5.3, 5.0	5.7
			13	6/12	50	5.8, 5.5, 5.0, 5.0, 4.5, 4.0	5.0
5/5	7.7, 7.1, 7.0, 6.9 6.9	7.0	11	2/12	17	5.0, 4.7	4.9
5/5	6.2, 6.1, 6.0, 6.0, 5.9	6.0	11	1/12	8	4.0	4.0
5/5	5.7, 5.1, 4.9, 4.5, 4.5	4.9	11	0/12	0	..	..
5/5	5.0, 4.2, 4.0, 3.8 3.3	4.0	11	0/12	0	..	..

* All titers expressed as the mouse $1/\log_{10}$ IC LD_{50}.

utes at 1500 r.p.m. and serial 10-fold dilutions were made in broth. Five 3-week-old mice were inoculated intracerebrally per dilution and the LD_{50}s calculated by the method of Reed and Meunch (1938).

RESULTS. The relationship between the virus ingested by *A. albimanus* mosquitoes and that present after 7 and 11 days of extrinsic incubation is shown in Table 1. The initial titers are for those mosquitoes sampled immediately after feeding on each of the serial 10-fold dilutions of EEE virus. The infection threshold on day 11 was equal to or less than 4.7 mouse $1/\log_{10}$ IC LD_{50} and the 50 percent infection threshold was betwen 6.9 and 8.0 mouse $1/\log_{10}$ IC LD_{50}. The positive EEE virus titers after 11 days ranged from 3.3 to 7.2.

The relationship between the virus ingested by *A. quadrimaculatus* and that present after 10, 11 and 13 days of extrinsic incubation is shown in Table 2. The infection threshold on day 11 was equal to or less than 6.0 mouse $1/\log_{10}$ IC LD_{50} and the 50 percent infection threshold was 7.6 mouse $1/\log_{10}$ IC LD_{50}. The positive EEE virus titers after 10 to 13 days ranged from 3.8 to 7.7.

The results of the transmission attempts are shown in Table 3. The median positive EEE virus titers shown are for all the mosquitoes which were allowed to feed. In the tests, a total of 11 *A. albimanus* and 2 *A. quadrimaculatus* transmitted the virus. The transmission threshold for the *A. albimanus* was between 6.0 and 6.9 mouse $1/\log_{10}$ IC LD_{50}. The titers of the transmitting *A. albimanus* mosquitoes ranged from 5.1 to 7.2. The transmission threshold for the *A. quadrimaculatus* was between 7.0 and 7.6 mouse $1/\log_{10}$ IC LD_{50}.

DISCUSSION. Similar studies with *Aedes aegypti* have been previously reported (Collins, *et al.*, 1965). A comparison of the infection of these three mosquitoes indicated that the *Ae. aegypti* and the *A. albimanus* had approximately the same infection threshold, 4.5 versus 4.7 mouse $1/\log_{10}$ IC LD_{50} after 11 days of extrinsic incubation. However, when low concentrations of EEE virus were used as an infective source, the *Ae. aegypti* were infected more often than the *A. albimanus*. The *A. quadrimaculatus* were less susceptible than the other species at all virus concentrations.

TABLE 3.—Relationship of initial EEE titer to virus transmission by *Anopheles albimanus* and *A. quadrimaculatus*.

Median Initial EEE Titer	Day	Trans./ Attempts	EEE virus titers	
			Median Pos.*	Transmitting Mosquitoes
		ANOPHELES ALBIMANUS		
8.0	7	2/14	5.8	6.0, 5.2
	11	8/19	6.0	7.2, 6.5, 6.3, 6.1, 6.0, 5.9, 5.8, 5.1
6.9	11	1/11	5.5	5.7
6.0	11	0/11	5.7	..
4.7	11	0/12	5.0	..
		ANOPHELES QUADRIMACULATUS		
7.6	10	2/20	4.8	5.5, 4.5
	11	0/12	5.7	..
	13	0/12	5.0	..
7.0	11	0/12	4.9	..
6.0	11	0/12	4.0	..
4.9	11	0/12	..	..

* Median positive EEE virus titer for all mosquitoes allowed to feed.

Although the transmission threshold for the *Ae. aegypti* mosquitoes was considerably lower than that of the *A. albimanus* and *A. quadrimaculatus* mosquitoes, with median initial EEE virus titers of 4.9 versus 6.9 and 7.6 respectively, the latter two species were definitely shown to be capable of experimentally transmitting EEE virus with the virus titers of the transmitting mosquitoes being approximately equal for all three species.

SUMMARY. *Anopheles albimanus* and *A. quadrimaculatus* mosquitoes were infected with eastern encephalitis (EEE) virus using the membrane feeding technique. Transmission of EEE virus to baby chicks was obtained with both species of mosquito. The *A. albimanus* were the more susceptible to infection and proportionately more capable of transmitting the infection. The infection thresholds for *A. albimanus* and *A. quadrimaculatus* were equal to or less than 4.7 and 6.0 mouse $1/\log_{10}$ IC LD_{50} respectively. The transmission thresholds for these mosquitoes were between 6.0 and 6.9 and between 7.0 and 7.6 mouse $1/\log_{10}$ IC LD_{50} respectively.

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LIST OF MOSQUITO RECORDS FROM ALBERTA

A. PUCAT

Department of Entomology, Macdonald College of McGill University, Macdonald College P.O., Quebec

The following forty-two species of mosquitoes, belonging to eight genera are known to occur in Alberta. The stages of mosquitoes recorded are indicated as larvae (L), adults (♂ ♀), and unspecified (u). These were collected mainly from the following general regions: (Numbers in parentheses refer to the correspondingly numbered references, which contain the original data.) Southern Alberta—McLintock (8), Shemanchuk (13), and Strickland (14). Central Alberta—Belur (1), Hocking (6), Klassen (7), Pucat (9), Strickland (14), and Wada (16). Northern Alberta—Happold (5), Hocking (6), and Pucat (9, 10). Twinn (15), Rempel (11, 12), Carpenter and La Casse (3), and Cook (4) refer mainly to Strickland's records.

Aedes (Ochlerotatus) campestris Dyar and Knab L (11, 13, 16); ♂ (7, 13); ♀ (1, 13); u (14, 15). *A. canadensis* Theobald L (9, 11, 16); ♂ (7, 9); u (14, 15). *A. cataphylla* Dyar L (5, 11, 13, 16); ♂ (7); ♀ (1, 5, 7, 9, 12, 13); u (14). *A. cinereus* Meigen L (5, 9, 11, 13, 16); ♂ 7, 9); ♀ (1, 5, 6, 7, 9, 13, 16); u (14, 15). *A. communis* de Geer L (5, 9, 11, 16); ♂ (7, 9); ♀ (5, 6, 7, 9, 16); u (14, 15). *A. diantaeus* Howard, Dyar